Application No.: 10/724,264 Attorney Docket No.: 02716.0005.NPUS01

## THE AMENDMENTS

## In the Claims:

1. (Currently Amended) A proteorhodopsin mutant having improved optical characteristics, said mutant is a proteorhodopsin variant comprising a mutation in a conserved histidine residue, said proteorhodopsin variant is of a naturally occurring proteorhodopsin or a proteorhodopsin homolog thereof having at least 90% identity with the naturally occurring proteorhodopsin, said conserved histidine is present at the position equivalent to position 75 of SEQ ID NO: 3 when the proteorhodopsin variant is aligned with SEQ ID NO: 3 for a maximum identity, wherein said proteorhodopsin mutant has lower p $K_{th}$  in comparison with the naturally occurring proteorhodopsin or the homolog thereof proteorhodopsin variant.

## 2-3. (Cancelled)

- 4. (Previously Presented) The proteorhodopsin mutant according to Claim 1, wherein said naturally occurring proteorhodopsin comprises SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, or 161.
- 5. (Currently Amended) The proteorhodopsin mutant according to Claim [[1]] <u>25</u>, wherein said <del>conserved histidine residue is at amino acid position 77 of <u>naturally occurring</u> <u>proteorhodopsin comprises</u> SEQ ID NO: 1 or <del>position 75 of</del> SEQ ID NO: 3.</del>
- 6. (Cancelled)
- 7. (Previously Presented) The proteorhodopsin mutant according to Claim 1, wherein said conserved histidine residue is mutated to an amino acid capable of forming a hydrogen bond.

Application No.: 10/724,264

Attorney Docket No.: 02716.0005.NPUS01

8. (Original) The proteorhodopsin mutant according to Claim 7, wherein said amino acid capable of forming a H-bond is asparagine, glutamine, lysine, arginine, tryptophan, serine, threonine, tyrosine, aspartic acid, or glutamic acid.

9. (Original) The proteorhodopsin mutant according to Claim 8, wherein said amino acid capable of forming an H-bond is asparagine, glutamine, lysine, tryptophan, aspartic acid, or glutamic acid.

10-12. (Cancelled)

- 13. (Withdrawn) An isolated nucleic acid sequence encoding the proteorhodopsin mutant according to Claim 1.
- 14. (Previously Presented) The proteorhdopsin mutant according to Claim 1, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 163, 165, 167, 169, 171, 173, 175, and 177.
- 15. (Withdrawn- Previously Presented) The isolated nucleic acid sequence according to Claim 13, selected from the group consisting of SEQ ID NOs: 164, 166, 168, 170, 172, 174, 176, and 178.
- 16. (Withdrawn- Currently Amended) A method for preparing the proteorhodopsin mutant having improved optical characteristics according to Claim 1, comprising the steps of:
  - (a) identifying [[a]] <u>the</u> conserved histidine amino acid residue of [[a]] <u>the</u> naturally occurring proteorhodopsin <u>or the proteorhodopsin homolog</u>,
  - (b) mutagenizing the conserved histidine amino acid residue, and obtaining proteorhodopsin mutants,
  - (c) determining the optical characteristics of the proteorhodopsin mutants, and
  - (d) selecting the proteorhodopsin mutant having improved optical characteristics.

Application No.: 10/724,264 Attorney Docket No.: 02716.0005.NPUS01

17-18. (Cancelled)

19. (Withdrawn) The method according to Claim 16, wherein said conserved amino acid residue is mutagenized by site-directed mutagenesis.

20. (Cancelled)

- 21. (Withdrawn) A method of storing and retrieving optical data, comprising the steps of:
  - (a) providing a film comprising a matrix having the proteorhodopsin mutant according to Claim 1 immobilized within,
  - (b) exposing the film to light of a wavelength that is absorbed by the proteorhodopsin mutant at a resting state in a predetermined pattern,
  - (c) converting selective portions of the film to an excited state and storing optical data therein,
  - (d) exposing the film of step (c) to light of a wavelength that is absorbed by the proteorhodopsin mutant at either a resting state or an excited state, and
  - (e) detecting the stored optical data by an optical recording device.
- 22. (Withdrawn) A light-driven energy generator comprising: (a) the proteorhodopsin mutant according to Claim 1, (b) a cell membrane, (c) a source of all-trans-retinal, and (d) a light source, wherein the proteorhodopsin mutant integrates within the cell membrane to produce an integrated proteorhodopsin mutant, and the integrated proteorhodopsin mutant binds covalently to all-trans-retinal to produce a light absorbing pigment.
- 23. (Previously Presented) The proteorhodopsin mutant according to Claim 1, wherein the <u>proteorhodopsin</u> homolog has at least 97% identity with the naturally occurring proteorhodopsin.
- 24. (Previously Presented) The proteorhodopsin mutant according to Claim 14, comprising the amino acid sequence of SEQ ID NO: 165.

Application No.: 10/724,264 Attorney Docket No.: 02716.0005.NPUS01

25. (Currently Amended) A proteorhodopsin mutant having improved optical characteristics, said mutant is a proteorhodopsin variant comprising a mutation in a conserved histidine residue, of said proteorhodopsin variant is a naturally occurring proteorhodopsin having a sequence selected from the group consisting of SEQ ID NO: 1, 3, 27, 103, 121, 125, 133, 139, 151, or 161, or a proteorhodopsin homolog thereof having at least 90% identity with the naturally occurring proteorhodopsin, said conserved histidine is present at the position equivalent to position 75 of SEQ ID NO: 3 when the proteorhodopsin variant is aligned with SEQ ID NO: 3 for a maximum identity, wherein said proteorhodopsin mutant has lower  $pK_{rh}$  in comparison with the naturally occurring proteorhodopsin or the homolog thereof proteorhodopsin variant.

- 26. (Currently Amended) The proteorhodopsin mutant according to Claim 25, wherein the <u>proteorhodopsin</u> homolog has at least 97% identity with the naturally occurring proteorhodopsin.
- 27. (Currently Amended) The proteorhodopsin mutant according to Claim 25, wherein the naturally occurring proteorhodopsin has comprises the amino acid sequence of SEQ ID NO: 3.